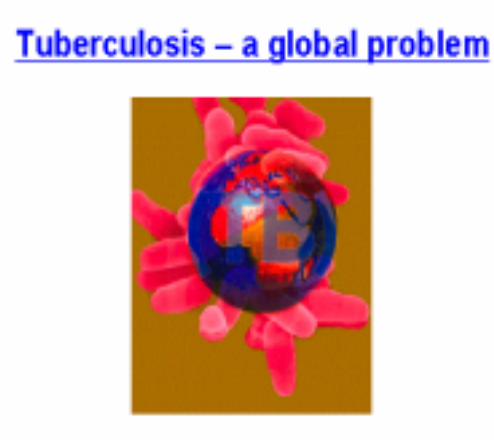
Measuring Enzyme Activity to Counter the Threat of Drug-Resistant Tuberculosis

Tuberculosis is a dreadful human disease caused by the M. tuberculosis bacterium and accounts for more causalities than any other single infection. It is also a potential bioterrorism agent. NIST scientists are studying the biochemical properties and three dimensional structure of chorismate mutase, an enzyme critical for the pathogen's survival. This enzyme is not found in humans, making it an attractive potential target for antibiotic development. This work is expected to enable innovation on new therapeutic approaches to treating this pathogen.



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Multi-drug resistant *Mycobacterium tuberculosis* is classified as a bioterrorism agent by the Center for Disease Control and Prevention and the National Institute of Allergy and Infectious Diseases. It is also a very robust natural human pathogen and has recently shown up in the U.S. This work is designed to understand chorismate mutase enzyme activity through selectively creating mutant enzymes that can me analyzed for its activity. By understanding the effects of the introduced mutations, one can determine where on the protein, antibiotics could interact to inhibit the enzyme and kill the bacterium.

Chorismate mutase is present only in bacterial and lower eukaryotic organisms but is absent in higher eukaryotes such as humans. Hence, this enzyme is a popular target in metabolic engineering and drug development. Chorismate mutase is a central enzyme in the shikimate pathway that is responsible for the synthesis of aromatic amino acids and a number of other key intermediates of commercial interest.

NIST scientists are developing genetic knockouts of an enzyme critical for the survival of the tuberculosis bacterium. This will enable precise determination of potential targets for the development of antibiotics.

Mycobacterium tuberculosis genome sequence revealed two genes for chorismate mutase, Rv1885c and Rv0948c. Rv1885c and Rv0948c chorismate mutase genes were amplified by polymerase chain reaction. Amplified genes were cloned into a protein expression vector. Both the recombinant plasmids were introduced into *E. coli* strain MZI for protein expression. Expressed chorismate mutases were purified to homogeneity and the biochemical properties of both chorismate mutases were studied. In addition, we determined the three dimensional crystal structure of Rv1885c chorismate mutase.

The gene Rv1885c from the genome of *Mycobacterium tuberculosis* encodes a 199 amino acid monofunctional and secreted chorismate mutase (*MtCM) with a 33 amino acid cleavable signal sequence, hence belongs to the *AroQ class of chorismate mutases. Consistent with the heterologously expressed *MtCM having periplasmic destination in *E. coli* and the absence of a discrete periplasmic compartment in *M. tuberculosis*, we observed that *MtCM secretes into the culture filtrate of *M. tuberculosis*. *MtCM functions as a homodimer and exhibits a dimeric state of the protein at as low as 5 nanomolar protein concentration. *MtCM exhibits simple Michaelis-Menten kinetics with a K_m of 0.5 ± 0.05 mM and a k_{cat} of 60 s^{-1} per active site (at 37 ^oC and pH 7.5). The crystal structure of *MtCM has been determined at 1.7 Å resolution (Protein Data Bank ID: 2F6L). The protein is all alpha helical and the active site is formed within a single chain without any contribution from the second chain in the dimer. Analysis of the structure shows a novel fold topology for the protein with a topologically rearranged helix containing Arg_{134}. We provide evidence by site directed mutagenesis that the residues Arg_{49}, Lys_{60}, Arg_{72}, Thr_{105}, Glu_{109}, and Arg_{134} constitute the catalytic site; the numbering of the residues includes the signal sequence. Our investigation on the effect of phenylalanine, tyrosine, and tryptophan on *MtCM shows that *MtCM is not regulated by the aromatic amino acids. Consistent with this observation, the x-ray structure of *MtCM does not have an allosteric regulatory site.

Future Plans: We are in the process of synthesizing a small molecule inhibitor. We will test for inhibition of chorismate mutase activity as well as the effect of the inhibitor on the growth of *Mycobacterium tuberculosis*. We hope this research would lead to new drug discovery for *Mycobacterium tuberculosis*.